

Chapter 15: Microorganisms on the CCL 2

A chapter from:

**Regulatory Determinations Support Document for Selected Contaminants
from the Second Drinking Water Contaminant Candidate List (CCL 2)**

EPA Report 815-D-06-007

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Abbreviations

AWWARF	American Water Works Association Research Foundation
CCL	Contaminant Candidate List
CCL 2	Second Contaminant Candidate List
CFU	Colony Forming Unit
DRINK	Drinking Water Research Information Network
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FISH	Fluorescent In Situ Hybridization
GAC	Granular Activated Carbon
HPLC	High Performance Liquid Chromatography
IARC	International Association for Research on Cancer
ICR	Information Collection Rule
LC/MS	Liquid Chromatography/ Mass Spectrometry
LPS	Lipopolysaccharide
MAC	Mycobacterium Avium Complex
PAC	Powdered Activated Carbon
PCR	Polymerase Chain Reaction
PPIA	Protein Phosphatase Inhibition Assay
RNA	Ribonucleic Acid
UCMR	Unregulated Contaminant Monitoring Regulation
UCMR 1	First Unregulated Contaminant Monitoring Regulation
UV	Ultraviolet
WHO	World Health Organization

15 Microorganisms on the CCL 2

The nine microbial contaminants listed on the Second Contaminant Candidate List (CCL 2) include:

- Four virus groups - Caliciviruses, Echoviruses, Coxsackieviruses, and Adenoviruses
- Four bacteria/bacterial groups - *Aeromonas hydrophila*; *Helicobacter pylori*; *Mycobacterium avium intercellulare* or MAC; and Cyanobacteria (blue-green algae), other fresh water algae, and associated toxins
- One group of protozoa - Microsporidia (*Enterocytozoon bienersi* and *Septata intestinalis*, now renamed *Encephalitozoon intestinalis*)

15.1 Evaluation of Microbial Contaminants for Regulatory Determination

In addition to considering if the Agency had sufficient information to address the three statutory criteria (i.e., adverse health effects, known/likely occurrence, and meaningful opportunity for health risk reduction), the Agency also considered whether sufficient information was available to determine the effectiveness of current treatment requirements for controlling the nine microbial contaminants. After consideration of these factors, the Agency determined that none of the nine microbial contaminants have sufficient information at this time to address the three statutory criteria or the question about whether current treatment practices adequately control for these organisms.

General areas where information is insufficient are identified in Exhibit 15-1. Section 15.2 briefly summarizes the available occurrence, health, analytical methods, and treatment information on the nine CCL 2 microorganisms. Section 15.3 provides a brief overview of ongoing research and data gaps.

Exhibit 15-1: Information Gaps for the CCL 2 Microbial Contaminants

Health Effects	Treatment	Analytical Methods	Occurrence
Microsporidia Some Cyanotoxins	<i>Aeromonas</i> MAC <i>Helicobacter</i> Adenoviruses Caliciviruses Coxsackieviruses Echoviruses Microsporidia Some Cyanotoxins	<i>Aeromonas</i> MAC <i>Helicobacter</i> Microsporidia Some Cyanotoxins	<i>Aeromonas</i> MAC <i>Helicobacter</i> Adenoviruses Caliciviruses Coxsackieviruses Echoviruses Microsporidia Some Cyanotoxins

15.2 Microbial Contaminant Profiles

15.2.1 *Helicobacter pylori*

Characteristics

Helicobacter pylori, first isolated from humans in 1983, is a spiral-shaped, microaerophilic, non-sporulating, gram-negative rod-shaped bacteria (CDC, 1999). Humans are the only known reservoir for *H. pylori*, which resides in the gastric mucous layer, or adheres to the epithelial lining of the stomach (Madigan *et al.*, 1997; CDC, 2002). In the United States, *H. pylori* is most prevalent among older adults, African Americans, Hispanics, and lower socioeconomic groups; about two-thirds of the world's population is infected (Staat *et al.*, 1996; CDC, 2002).

Health Effects

Peptic ulcer disease, gastric cancer, and gastric lymphoma are the classic health effects ascribed to *H. pylori* infection, but clinical manifestation may also include non-ulcer dyspepsia, and gastro-esophageal reflux disease (Vassili and Malfertheiner, 2003; Isakov and Malfertheiner, 2003). Recent evidence also suggests a possible association of cardio- and cerebrovascular diseases, hematologic disease, skin diseases, intractable nausea during pregnancy, and hepatobiliary disease with *H. pylori* infection (Gasbarrini *et al.*, 2003; Zuberbier, 2003; Diaz *et al.*, 2003). The International Association for Research on Cancer (IARC) has classified *H. pylori* as carcinogenic to humans (group 1) (IARC, 1994).

Analytical Methods

H. pylori is difficult to culture. A selective medium that discriminates it from background bacteria and a reliable method to detect viable organisms are under development (Degnan *et al.*, 2003). Molecular methods, such as fluorescent in situ hybridization (FISH) and the polymerase chain reaction (PCR), can aid in the detection of *H. pylori* but they fail to distinguish viable and non-viable organisms (Van Doorn *et al.*, 2000; Moreno *et al.*, 2003).

Occurrence and Exposure

The organism's only natural ecological niche is the stomach lining of humans. Evidence of this pathogen's survival in the environment is limited due to difficulty in culturing. Using molecular techniques, researchers have detected *H. pylori* in ambient water, including some drinking water sources, in the U.S., Canada, Japan, and Sweden (Hegarty *et al.*, 1999a; McKeown *et al.*, 1999; Sasaki *et al.*, 1999; Hulten *et al.*, 1998). More research is needed on the occurrence of viable *H. pylori* in drinking water, including ground water sources that may be affected by ambient surface waters. *H. pylori* is included on List 3 of EPA's Unregulated Contaminant Monitoring Rule (UCMR) (64 FR 50556). Monitoring will begin when a suitable analytical method has been developed and tested.

H. pylori has a low infective dose and a high prevalence in human populations. Seroprevalence studies indicate that more than 50 percent of people in the United States are

infected, although this rate is declining (Staat *et al.*, 1996). Some epidemiological data on *H. pylori* suggest host-to-host transmission, although common sources of infection, such as food or drinking water, have also been implicated (Malaty *et al.*, 1991; Blecker *et al.*, 1994). The likelihood of waterborne transmission in the U.S. has not been determined, but it is a strong risk factor in developing countries (Hegarty *et al.*, 1999b). Most infections are acquired in childhood, although children may remain asymptomatic (Lanciers *et al.*, 1996; Rowland and Drumm, 1998).

Water Treatment

Studies indicate that individual cases of *H. pylori* are readily inactivated by chlorine and removed by conventional and membrane filtration (Johnson *et al.*, 1997; Gerba *et al.*, 2003a). However, there is uncertainty about the survival rates of the non-culturable coccoid form. Also, there is still a question about the disinfection efficiency of disinfectants other than chlorine, especially for aggregated or adsorbed cells (Baker *et al.*, 2002).

15.2.2 Aeromonas hydrophila

Characteristics

Aeromonas hydrophila is a gram-negative, oxidase-positive, non-spore-forming, facultatively anaerobic rod, some strains of which are of clinical importance. *A. hydrophila* is free-living in soil and water, and is not necessarily associated with fecal contamination. It grows in the biofilm of distribution systems.

Health Effects

Aeromonas species can cause gastroenteritis and infection of wounds (Smith and Cheasty, 1998; Janda and Abbott, 1998; Vila *et al.*, 2003). Diarrhea is generally self-limited and lasts a few days to a few weeks (Tomar, 2001). Chronic diarrhea of more than a few weeks has been described in children under the age of five years who have predisposing conditions such as treatment with antibiotics to which Aeromonads are resistant (Moyer, 1987). Disseminated, systemic disease in the compromised hosts (especially those who have underlying liver disease or cancer) causes a high fatality rate. Other systemic infections include bacteremia, septicemia, cirrhosis, and endocarditis (Lau *et al.*, 2000; Chang *et al.*, 1997; Braun *et al.*, 2001; Brouqui and Rault, 2001).

Exposure to *Aeromonas* occurs through ingestion of contaminated food and water, and dermal contact with water or soil. Person-to-person transmission is rare (Farmer *et al.*, 1992; Janda and Abbott, 1998). Transmission generally occurs by exposure to *Aeromonas* in aqueous environments, either via trauma or wound infection or by ingestion (Altwegg and Geiss, 1989; Esteban *et al.*, 1999). Exposure to surface water is the primary risk factor associated with wound infection. No waterborne outbreaks have been reported.

Analytical Methods

EPA Method 1605 uses a selective medium to distinguish species and strains of *Aeromonads* in water (USEPA, 2000). This was the method used for screening under the UCMR. One drawback of this method is that it only identifies to the genus level and thus does not differentiate between pathogenic and non-pathogenic strains (Altwegg *et al.*, 1990). Several molecular methods are available but none have been standardized for general use (Peng *et al.*, 2002; Wang *et al.*, 2003).

Occurrence and Exposure

A. hydrophila has been found in all types of water: wastewater, surface water, ground water, marine and estuarine environments, and even chlorinated water supplies (Pettibone, 1998; Borrell *et al.*, 1998; Bianucci *et al.*, 2001). It grows in distribution systems (Gavriel *et al.*, 1998; Smith and Cheasty, 1998). EPA included *Aeromonas* as an analyte in the recent UCMR Screening Survey (2001-2003). Under this survey, drinking water samples were collected from 300 water systems, 6 times a year, and at 3 different sampling locations per system. The samples were analyzed using EPA Method 1605 (USEPA, 2000). *Aeromonas* was detected in 2.6% of the samples. (For details, see the forthcoming report entitled *The Analysis of Occurrence Data from the First Unregulated Contaminant Monitoring Regulation (UCMR 1) in Support of Regulatory Determinations for the Second Drinking Water Contaminant Candidate List.*) Further work is needed to determine the pathogenicity of isolates detected in these samples.

Water Treatment

Standard water treatment with chlorine disinfectant appears to reduce the numbers of *Aeromonads* to levels below 1 colony forming unit (CFU) per 100 ml (Nichols, 1996). However, treated water can support *Aeromonad* growth in storage reservoirs or distribution systems, and *Aeromonads* can contribute to biofilm, which makes them more resistant to disinfectant residuals (Gavriel *et al.*, 1998). The concentration and rate of growth depend on temperature, organic content of the water, residence time in the distribution system, and the amount of residual chlorine disinfectant (Burke *et al.*, 1984).

15.2.3 *Mycobacterium avium* Complex (MAC)

Characteristics

MAC is a group of slow-growing, gram-positive, aerobic, rod-shaped bacteria that consists of two predominant species, *Mycobacterium intracellulare* and *Mycobacterium avium*, the latter of which has three subspecies: *M. avium* ssp. *avium*, *M. avium* ssp. *paratuberculosis*, and *M. avium* ssp. *silvaticum* (Thorel *et al.* 1990a, 1990b; Inderlied *et al.* 1993; Cangelosi *et al.* 2003). These bacteria are ubiquitous in the environment and cause opportunistic infections in humans and animals (Falkinham, 1996). Due to the low permeability of their cell walls, MAC are resistant against therapeutic agents (Minnikin, 1991; Nikaido *et al.*, 1993). Mycolic acids, complex lipids located within the cell wall, contribute to the hydrophobic nature of the cell envelope and are thought to play a role in the organism's resistance against therapeutic agents (Nikaido *et al.*, 1993).

Health Effects

MAC infections occur most often in immunocompromised individuals (Horsburgh, 1991). Infections caused by MAC include cervical lymphadenitis (inflammation of neck lymph nodes), joint infections, pulmonary infections and bacteremia (Swanson *et al.*, 1998; Wolinsky, 1995; Horsburgh *et al.*, 1992). MAC-related pulmonary disease typically occurs in patients with impaired cellular immunity or chronic lung disease (Aksamit, 2002). MAC usually manifests in AIDS patients as a disseminated disease involving the lungs, lymph nodes, and gastrointestinal tract. Mycobacterial cervical lymphadenitis has long been recognized as a disease of children between 6 months and two years of age; infection is limited to the cervical and mandibular lymph nodes. *M. paratuberculosis* is suspected in the etiology of Crohn's Disease (Romero *et al.*, 2005). EPA established a Health Advisory for mycobacteria in 1999 (USEPA, 1999).

Analytical Methods

Culture and isolation of MAC bacteria from environmental samples is problematic because of their slow growth (it could take months to see colonies on plates), particular nutrient requirements, and the presence of other microorganisms that quickly outgrow MAC. The use of nucleic acid probes for group or species determination has generally replaced biochemical identification methods for screening samples. Definitive identification of mycobacteria is possible by determination of methylated fatty acid or through examination of mycolic acid profiles by chromatographic methods (Ozbek and Aktas, 2003; HPLC Users Group, 1999). PCR methods are available to facilitate direct detection of MAC in environmental samples. Molecular typing methods have demonstrated remarkable heterogeneity among MAC strains; typing has shown both taxonomic and epidemiological value (van Soolingen, 2001).

Occurrence and Exposure

MAC has been isolated from tap water samples worldwide (Aronson *et al.*, 1999; Covert *et al.*, 1999). The presence of these bacteria in tap water is attributed to their high resistance to disinfectants commonly used in water treatment and their ability to grow in biofilms in distribution systems (von Reyn *et al.*, 1993, 1994). MAC is thermo-tolerant (it grows at temperatures in the range of 52 to 57 °C), and has frequently been isolated from recirculated hot water systems in institutions such as hospitals (Embil *et al.*, 1997; Kahana and Kay, 1997). Increased zinc levels have been suggested to favor growth and survival of MAC. Some hospitals use galvanized pipes made with zinc alloys which would contribute to MAC growth. In several studies, MAC has been isolated from biofilm in water distribution systems in the U.S., indicating that biofilms may be a significant reservoir for the organism (Norton *et al.*, 1999; Falkinham *et al.*, 2001).

Water Treatment

MAC bacteria exhibit significant resistance to chlorine, chloramine, chlorine dioxide, and ozone disinfection of drinking water (Taylor *et al.*, 2000; Le Dantec *et al.* 2002; Gerba *et al.*, 2003a). This is probably due to the presence of waxy material in their cell wall and the ability of some strains to clump together (Gerba *et al.*, 2003a). However, because mycobacteria adhere to

particles, a large fraction are removed from drinking water by flocculation, sedimentation, and filtration. They are also sensitive to ultraviolet radiation (USEPA, 2002).

MAC bacteria have been isolated from drinking water distribution biofilms (Iivanainen *et al.*, 1999; Falkinham *et al.*, 2001). The fact that colonization of water distribution systems occurs in both surface and ground water sources suggests that mycobacteria in the distribution system might represent renewable biofilm (Falkinham, 2002).

15.2.4 Cyanobacteria (blue-green algae), other fresh water algae, and their toxins

Characteristics

Cyanobacteria, often referred to as blue-green algae, more closely resemble bacteria than algae. Of the thousands of cyanobacterial species that are known to exist, an estimated 46 of them produce toxins (WHO, 1998). Cyanobacterial toxins are divided into three groups: cyclic peptides, alkaloids, and lipopolysaccharides (LPSs). The toxins of most concern in the United States are microcystin, which is a cyclic peptide, and cylindrospermopsin, anatoxin-a, saxitoxin, and anatoxin-a(s), which are all alkaloids (USEPA, 2001).

Health Effects

Most recognized cyanotoxins are hepatotoxins or neurotoxins. Cyanobacterial toxins can also cause skin irritation, acute gastroenteritis, and possibly cancer (Chorus and Bartram, 1999). Illnesses have been reported following ingestion of contaminated water, and in at least one case deaths occurred after hemodialysis treatments that used water contaminated with cyanotoxins (130 patients became ill, and 50 of them died). Biological and chemical evidence pointed to the occurrence of microcystins in treatment water as being the major factor in these deaths (Carmichael *et al.*, 2001; Pouria *et al.*, 1998; Jochimsen *et al.*, 1998; Chorus and Bartram, 1999). Children and immunocompromised people may be at greatest risk of health effects from cyanobacterial toxins (Pilotto *et al.*, 1999). The World Health Organization (WHO, 2004) has set a provisional guideline value of 1 µg/L for microcystin-LR.

Analytical Methods

Several analytical methods are available for detecting cyanobacteria and their toxins in drinking water. Some research methods currently available for some toxins include high performance liquid chromatography (HPLC), enzyme linked immunosorbent assay (ELISA), protein phosphatase inhibition assay (PIIA), and liquid chromatography/mass spectrometry (LC/MS). However, none of these methods have been standardized (USEPA, 2001).

Occurrence and Exposure

Cyanobacteria are ubiquitous in the aquatic environment. They occur in surface water whenever nutritional, temperature, and water flow conditions are favorable for their growth (Horne and Goldman, 1994; Yoo *et al.*, 1995; Chorus and Bartram, 1999). Some cyanobacteria release toxins throughout their life cycle, while others only release toxins upon cell death or lysis (Yoo *et al.*, 1995). When released to the aquatic environment, cyanobacterial toxins may persist

for several weeks to months (Yoo *et al.*, 1995; Chorus and Bartram, 1999). The American Water Works Association Research Foundation (AWWARF, 2000) performed a preliminary monitoring study of microcystins in drinking water using a screening method. The result showed that 80% of the samples (539 out of 677) collected by participating U.S. and Canadian water utilities were positive for microcystins but only 4.3% of the positive samples had concentrations higher than WHO's 1 µg/L guideline. A recent Wisconsin study showed microcystin levels to be as high as 6 µg/L in some raw waters (USEPA, 2001). At this time there is no national database on the occurrence and frequency of cyanobacterial blooms or their toxins in the United States.

Water Treatment

Controlling source water to make it unsuitable for algal growth is one of the most effective methods of water treatment (Burns, 2000; USEPA, 2001). Riverbank filtration also shows promise for toxin removal (Holst *et al.*, 2003). Water treatment techniques (coagulation, sedimentation, filtration, disinfection, granular activated carbon (GAC), powdered activated carbon (PAC), ozonation, and ultraviolet radiation) are effective to varying degrees at removing most of the most common cyanobacteria and their toxins in drinking water (USEPA, 2001; Burns, 2000; Hitzfeld *et al.*, 2000). When the appropriate combination of techniques is used, close to 100 percent of particular toxins can generally be eliminated in finished water, though algal blooms and high organic loads can limit treatment effectiveness (Drikas *et al.*, 2001; Karner, *et al.*, 2001; Hitzfeld *et al.*, 2000). Some treatment options (e.g., copper sulfate application) can cause cell lysis and produce an increase in extracellular toxin concentrations (Yoo *et al.*, 1995; USEPA, 2001).

15.2.5 Adenoviruses

Characteristics

Adenoviruses are double-stranded deoxyribonucleic acid (DNA) viruses. These are the largest of the viruses on the Contaminant Candidate List (CCL). They range in size between 60 and 90 nm (Foy, 1997). Adenoviruses were first noted in human adenoid and tonsil tissue in 1953 (Rowe *et al.*, 1953). More than 50 adenovirus types exist (De Jong *et al.*, 1999). Different types cause a wide range of health effects. Because adenoviruses can occur in human feces (Pina *et al.*, 1998), there is a potential for waterborne transmission. Adenovirus types with the greatest such potential are the enteric adenoviruses, types 40 and 41 (Foy, 1997).

Health Effects

Children under two years old are especially vulnerable to enteric adenovirus infection (LeBaron *et al.*, 1990). The immunocompromised are another sensitive subpopulation for adenoviruses (Hierholzer, 1992). Adenovirus infection can be present in the immunocompromised as a disseminated disease, affecting many different parts of the body. Adenoviruses can cause respiratory tract infection, pharyngitis, conjunctivitis, cystitis, gastroenteritis and other effects (Foy, 1997). Other serious health effects include Reyes Syndrome (Edwards *et al.*, 1985) and myocarditis (Pauschinger *et al.*, 1999). Adenoviruses have also been associated with weight gain in animals (Dhurandhar *et al.*, 2000).

Analytical Methods

No standardized and validated method exists for detecting adenoviruses in water. Adenoviruses grow in tissue culture cells, although the enteric adenoviruses, types 40 and 41, require different cell lines from the other adenovirus types. Adenoviruses grow slowly and may be overgrown by other viruses in mixed samples (Hurst *et al.*, 1988). A variety of molecular techniques involving PCR amplification have been used for detection and identification of adenoviruses (Chaperon *et al.*, 2000; Jiang *et al.*, 2001; Loge *et al.*, 2002).

Occurrence and Exposure

Adenoviruses are among the most commonly detected virus types in environmental waters (Chaperon *et al.*, 2000). Adenovirus infection by exposure to recreational water is well documented (D'Angelo *et al.*, 1979; Turner *et al.*, 1987; Papapetropoulou and Vantarakis, 1998). However, no cases of infection via drinking water have been reported. In some cases, researchers have observed seasonal variation in the frequency of diseases caused by adenoviruses (Krikelis *et al.*, 1985; Tani *et al.*, 1995). Adenoviruses appear to survive better in some water types than other viruses. Researchers have speculated that the greater longevity of adenoviruses in water may be due to their reliance on DNA, which is more stable than ribonucleic acid (RNA) (Enriquez *et al.*, 1995).

Water Treatment

Adenoviruses can be controlled by chlorine disinfection in laboratory studies (Gerba, 2003a). Aggregation of virus and adherence to particles can reduce the effectiveness of chlorine disinfection (Payment *et al.*, 1985). Adenoviruses are much more resistant to ultraviolet (UV) irradiation than other viruses (Gerba *et al.*, 2003a).

15.2.6 Caliciviruses

Characteristics

Caliciviruses are viruses that belong to the family *Caliciviridae*. The first documented outbreak of Norwalk virus, the best-known of the caliciviruses, occurred in Norwalk, Ohio in 1968 (Adler and Zicki, 1969). The Norwalk virus and related strains, known collectively as noroviruses, present a potential health risk in drinking water in the United States. The noroviruses are single-stranded RNA viruses (Jiang *et al.*, 1990). They are about 25 to 35 nm in diameter. Noroviruses occur in feces and have caused many waterborne disease outbreaks.

Health Effects

Human caliciviruses, including noroviruses, cause a self-limiting gastroenteritis which usually lasts 24 to 48 hours. Symptoms include vomiting, abdominal cramps, and diarrhea (MMWR, 1990).

Analytical Methods

No cell culture method for noroviruses exists (Atmar and Estes, 2001). Thus, viable noroviruses can't be enumerated in water or in treatment studies. Molecular detection methods are available, but no molecular method has yet been standardized for detection of noroviruses in water.

Occurrence

Occurrence information for noroviruses in water is limited due to the inadequacies of analytical methods. Nonetheless, noroviruses have been linked to many waterborne disease outbreaks in the U.S. (Kaplan *et al.*, 1982; Parshionikar *et al.* 2003). They have been detected in sewage effluent (Lodder *et al.*, 1999) and in ambient water (Griffin *et al.*, 1999).

Water Treatment

Studies of the susceptibility of human caliciviruses to water treatment are limited by the lack of culture methods for detecting viable viruses. Nevertheless, Shin *et al.* (1998) determined that Norwalk virus was much less resistant to chlorine inactivation than poliovirus, and was inactivated about as rapidly as MS2 bacteriophage. The small size of noroviruses may make them difficult to remove by filtration, unless they are aggregated with larger particles.

15.2.7 Echoviruses and Coxsackieviruses

Characteristics

Echoviruses and coxsackieviruses are single-stranded RNA viruses that are between 27 and 30 nm in diameter. Both viruses belong to the genus *Enterovirus*. Echoviruses were first isolated from cell cultures in the late 1940s. Coxsackieviruses were first isolated from patients with polio-like symptoms in Coxsackie, New York in 1948. There are at least 31 human echoviruses and 29 human coxsackieviruses. These viruses are transmitted through the fecal-oral route.

Health Effects

Coxsackieviruses and echoviruses first infect the intestinal tract, with or without symptoms (Modlin, 1986; Minor, 1998). After entering the blood stream, different serotypes of the viruses can infect most other organ systems. They may cause febrile illness, aseptic meningitis, respiratory disease, encephalitis, paralytic disease, and other effects (Melnick, 1997). Some serotypes can cause myocarditis (Martino *et al.*, 1995). Others are suspected of causing diabetes (Bantvala *et al.* 1985; Craighead, 1975; Notkins, 1977). Echovirus and coxsackievirus infections are among the most common viral infections of humans in the United States (CDC, 1997).

Analytical Methods

The Information Collection Rule (ICR) method is a standardized and validated method for the detection of culturable echoviruses and coxsackieviruses. However, the ICR method does not differentiate virus types. A PCR-based method can be used to differentiate coxsackieviruses and echoviruses detected in water (Vivier *et al.* 2001). However, this method does not necessarily indicate which viruses are infective. A hybrid method, the integrated cell culture/PCR-based method described by Reynolds *et al.* (2001), demonstrates viability through cell culture, but also gives PCR detection.

Occurrence

Coxsackieviruses and echoviruses are shed in feces and occur in sewage and contaminated waters. These viruses have been detected in ambient water (Vivier *et al.*, 2001), ground water (Powell *et al.*, 2003), and drinking water (Vivier *et al.* 2004). Waterborne outbreaks of disease caused by enteroviruses are not common, but have been documented (Hejkal *et al.* 1982).

Water Treatment

Hurst (1991) reported that physical water treatment with disinfection was effective in removing enteroviruses. Studies by Sobsey *et al.* (1988), Englebrecht *et al.* (1980), Payment *et al.* (1985), and others suggest that both echoviruses and coxsackieviruses are susceptible to chlorine disinfection. Nonetheless, culturable enteroviruses have been detected in treated water (Vivier *et al.*, 2004). Also, questions remain about the effectiveness of disinfection against particle-bound viruses, since the particles can afford some protection for viruses (Hejkal *et al.*, 1981).

15.2.8 Human Microsporidia: *Enterocytozoon bieneusi* and *Encephalitozoon* (formerly *Septata*) *intestinalis*

Characteristics

Microsporidia were first recognized as a distinct group of microorganisms in 1882 (Wittner, 1999). Microsporidia were first reported as human pathogens in 1959 (Matsubayashi *et al.*, 1959), and since 1985 they have emerged as pathogens of concern in immunocompromised patients (Desporte *et al.*, 1985). The two species of greatest concern for the immunocompromised are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. Microsporidia have an environmentally resistant stage, the spore, which gets its survival characteristics from its impervious spore wall. The spore is the infectious stage for new hosts. The spores of the human microsporidia species of concern are very small, in the range of 1 to 3 µm, as compared to *Cryptosporidium* spp. oocysts, which are 4 to 6 µm (Franzen and Müller, 1999).

Health Effects

Microsporidiosis primarily affects individuals who are immunocompromised, especially those infected with HIV (Bryan, 1995). Weiss and Keohane (1997) estimated that as many as 50% of individuals infected with HIV suffer from microsporidiosis. However, Kotler and Orenstein (1999) find that the recent success of HIV-control drugs in boosting immune function has greatly reduced the incidence of microsporidiosis in HIV-infected patients.

In the immunocompromised, microsporidia infect the gastrointestinal tract, causing diarrhea (Hutin *et al.*, 1998). They may also become disseminated throughout the body in these patients, causing a variety of adverse health effects (Orenstein *et al.*, 1997). Microsporidiosis appears to be uncommon in the immunocompetent. When it does occur, the most common effect in the immunocompetent appears to be a self-limiting diarrhea (Bryan *et al.*, 1997). Cases of traveler's diarrhea have been attributed to microsporidiosis (Fournier *et al.*, 1998; Sobottka *et al.*, 1995).

Analytical Methods

There is no standardized and validated method for detecting human microsporidia in environmental waters or drinking water. Research methods have been used to detect microsporidia in surface waters (Sparfel *et al.*, 1997; Fournier *et al.*, 2000) and ground waters (Dowd *et al.*, 1998). However, these methods have limitations. Methods involving water concentration, as described for example by Sparfel *et al.* (1997) and Borchardt and Spencer (2002), are not widely available. Due to the small size and lack of internal structure of microsporidia, direct microscopic detection is not a viable option. Methods that have been used in various microsporidia studies include PCR (Sorel *et al.*, 2003), real-time PCR (Menotti *et al.*, 2003), and fluorescent in-situ hybridization (Hester *et al.*, 2000). Development of analytical methods for *E. bienersi* is hindered by the lack of a culture method capable of producing spores in sufficient quantities for testing. A culture method is available for *Encephalitozoon* species (Bouladoux *et al.*, 2003).

Occurrence and Exposure

Due to the fecal and urinary mode of shedding of microsporidian spores (Orenstein *et al.*, 1992), waterborne transmission is possible. The spores of microsporidia can survive for weeks or months, and some can also survive drying (Kramer, 1970; Maddox, 1973; Undeen *et al.*, 1993). *E. bienersi* and *E. intestinalis* species have been detected in sewage (Franzen and Müeller, 1999), surface water (Sparfel *et al.*, 1997), ground water (Dowd *et al.*, 1998), and irrigation water (Thurston *et al.*, 1999). They have not been detected in drinking water. *E. bienersi* and *E. intestinalis* have been found in a variety of domestic and wild animals (Rinder *et al.*, 1997). In human hosts, there is no evidence to indicate a seasonality of microsporidiosis (Conteas *et al.*, 1998).

Water Treatment

Data on the effectiveness of water treatment for controlling *E. bienersi* are limited due to the lack of a culture method for this organism. Since *Encephalitozoon* species are culturable,

their susceptibility to treatment has been studied. In a model treatment plant, conventional physical treatment provided 2.47 log removal of *E. intestinalis* (Gerba *et al.*, 2003b). *E. intestinalis* is somewhat resistant to chlorine, but can nonetheless be controlled (Wolk *et al.*, 2000). *E. intestinalis* is also susceptible to UV radiation and ozone (Naumovitz *et al.*, 1998).

15.3 On-Going Research Activities at EPA to Overcome Data Gaps for the CCL 2 Microorganisms

EPA supports an active research program on the CCL 2 microorganisms to fill information gaps. For the design of treatment studies on the CCL viruses or surrogates, EPA emphasizes the need to conduct tests under realistic conditions, e.g., conditions where viruses might be protected by aggregating or adhering to particles. EPA believes it is important to conduct virus removal/inactivation studies in drinking water treatment plants or pilot plants. EPA is also pursuing method development for viruses to support these treatment studies.

EPA has completed a one-year UCMR survey of the genus *Aeromonas* in 292 public water systems. Researchers are still working on ways to characterize clinical strains and distinguish them from non-pathogenic strains, and developing methods to detect *Aeromonas* virulence factors. Similarly, researchers have conducted drinking water surveys for MAC, but they are still working on refining analytical methods and characterizing virulence factors. Methods for *H. pylori* are under development.

EPA is currently investigating the susceptibility of microsporidian *E. intestinalis* to chlorine and chloramine. In addition, EPA is sponsoring methods-related work on fluorescent gene probes, real-time PCR, concentration methods, and immunomagnetic separation for microsporidia. As part of ongoing environmental monitoring, researchers recently confirmed the presence of microsporidia in ground water. Researchers also participated in a workshop to assess the status of work on microsporidia.

EPA has funded projects on the removal of algal cells and cyanotoxins in a pilot-scale treatment plant, and on the effect of disinfection on cyanotoxins. EPA has developed analytical chemistry cyanotoxin standards, and is currently developing analytical methods for potential use in future monitoring. EPA has conducted several occurrence surveys for cyanotoxins and also a number of health effects studies. Researchers are currently conducting risk assessments to determine reference doses for the cyanotoxins. EPA has organized and participated in several workshops on cyanotoxins to assess the state of the science. EPA is taking steps to establish infective doses for CCL microbial contaminants through a formal review process.

For further information on these projects, please refer to the EPA's Office of Ground Water and Drinking Water Research Information Network (DRINK), found at www.epa.gov/safewater/drink/intro.html, a publicly accessible, web-based system that tracks over 1,000 ongoing research projects.

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